Quantification of Cutaneous Sensory Nerves and Their Substance P Content in Psoriasis

Anita Naukkarinen, Ph.D., Brian J. Nickoloff, M.D., Ph.D., and Eugene M. Farber, M.D.

Psoriasis Research Institute, Palo Alto, California (AN,EMF); Department of Pathology, Stanford University Medical Center, Stanford, California (AN); and Department of Pathology and Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, U.S.A. (BJN)

The aim of the present study was to extend our previous hypothesis that the inflammatory reaction in psoriasis is neurogenic, and that substance P mediates the inflammation. For this purpose, the pattern of neurofilament-positive sensory nerve fibers was studied and the lengths and substance P content of these fibers measured morphometrically in dermal and epidermal compartments of the psoriatic lesion, psoriatic but lesion-free skin, and control skin.

The epidermis and dermis of the psoriatic lesions were significantly more densely innervated with neurofilament-positive fibers than either lesion-free psoriatic or control skin. Although substance P is known to be rapidly degraded in tissues, and its actual concentrations in the sections were unknown, there was an increase in substance P containing nerves in the psoriatic lesion, the increase being significant in the epidermal nerve fibers. No significant differences in the measured parameters were obtained between lesion-free psoriatic and control skin.

These results indicate that there is an altered pattern of sensory nerves in a psoriatic plaque and that substance P may be an important mediator in the inflammatory processes that contribute either to the initiation or maintenance of a psoriatic lesion. *J Invest Dermatol 92:126–129, 1989*

Psoriasis is a disorder of the total skin appearing in patients genetically predisposed to this disease [1]. Histologically, psoriatic lesions are characterized by hyperplasia and altered differentiation of the epidermis, dilated capillaries in the papillary dermis, and inflammation in both dermis and epidermis [2]. The disease is known to have exacerbations and remissions affected by general changes occurring in the body, e.g., hormonal changes at puberty and menopause [3], and various forms of stress [4,5]. This suggests the involvement of neural-derived factors in the pathogenesis of psoriatic lesions. The symmetrical appearance of the lesions further supports this view [5]. When the role of the nervous system in inflammatory responses was studied, substance P (SP) was the first neuropeptide transmitter identified and purified. SP is synthesized in the dorsal root ganglia and transported to peripheral sensory nerve endings where it is released by various stimuli [6]. The skin is richly innervated with unmyelinated sensory fibers, and SP has been localized in the free nerve endings in the dermal papillae and epidermis of healthy human skin [7,8]. SP is the only effective neuropeptide shown to induce histamine release from rat mast cells [9]. Cooperation of histamine and SP is suggested to result in the axon reflex, i.e., activation of sensory nerves leads to peripheral release of SP, which in turn releases histamine from mast cells, and the released histamine further excites other sensory neurons [10]. In an early psoriatic lesion, one of the first morphologic changes observed by some investigators is the degranulating mast cell [11–13]. Mast cells are also more frequent in psoriatic lesions than in normal skin [14]. Thus, elements for neurogenic inflammation seem to exist in a psoriatic lesion. In fact, abnormality and increased turnover of cutaneous nerves in psoriasis has been previously reported by Weddell et al [15].

The aim of the present study was to find out if the innervation pattern and the content of SP in the cutaneous nerves are altered in psoriatic skin. For this, sections from psoriatic lesions, lesion-free psoriatic, and control skin biopsies were stained immunohistochemically and the nerve lengths measured morphometrically.

MATERIAL AND METHODS

Biopsies Punch biopsies of 3 to 4 mm were taken from lesional skin of 15 psoriatic patients, lesion-free skin of seven psoriatic patients, and control skin of nine persons. All the psoriatic patients were adults representing both sexes and different age groups. The psoriatic biopsies were at different stages from non-digital skin. Psoriatic, but lesion-free skin was taken at least 2 cm in distance from a lesion. One lesional and one lesion-free biopsy were taken from the same psoriatic patient. Otherwise all the biopsies studied were from different patients. Control skin was received from face lift operations, all the donors being female (54.7 ± 4.6 years, M ± SEM). Because site-matched biopsy material from healthy controls was not available, the face-lift skin was used because it has a rich neurovascular supply. After removal, the biopsies were immediately immersed in saline, and within a few hours embedded in OCT Compound (Miles Scientific, Naperville, IL) and frozen in liquid nitrogen. The frozen biopsies were stored in −70°C until further processed.

Immunohistochemical Stains For immunohistochemical staining, 16 μm cryosections were cut and placed on poly-L-lysine (Sigma Chemical Co., St Louis, MO) coated slides. Serial sections from the same biopsy were stained with both the polyclonal rabbit antihuman neurofilament (NF) antibody (gift from Dr. Dahl, West

Manuscript received January 14, 1988; accepted for publication July 22, 1988.

This study was financed by The Psoriasis Research Institute and The National Science Foundation

Part of this work has been presented at the World Congress of Dermatology, Berlin, May 24-29, 1987.

Reprint requests to: Anita Naukkarinen, Ph.D., Department of Clinical Pathology, Kuopio University Central Hospital, 70210 Kuopio-Finland

0022-202X/89/$03.50

Roxbury Veterans Administration Medical Center, Boston, MA), and the polyclonal rabbit anti-human substance P (SP) antibody (Histo-Tek Antisera, Miles Scientific). The anti-NF antibody was raised in rabbits against degraded antigen from phosphate buffer extracts of autolyzed human spinal cord as discussed by Dahl and Bignami [16]. This antiserum has been previously characterized in identification of sensory nerves in human skin [17]. The stainings were carried out at room temperature. After a 10 min treatment with normal goat serum, both antisera were used as a 1 : 1,000 dilution on sections for 60 min. After a thorough rinse with phosphate-buffered saline (PBS) a biotinylated goat anti-rabbit IgG (Vectastain ABC kit, PK-4001, Vector Laboratories, Burlingame, CA) was applied on the sections for 30 min. After PBS rinses and a 5 min hydrogen peroxide treatment an avidin-biotin-peroxidase complex (Vectastain ABC kit, PK-4001) was added on the sections for 40 min, followed by PBS rinses. The peroxidase enzyme was visualized by incubating the sections in 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Polysciences Inc., Warrington, PA) containing 0.04% nickel chloride and 0.03% hydrogen peroxide (Merck, Darmstadt, Germany) for 5 min. Before coverslipping the sections were washed under running tap water for 3 min, dehydrated in ethanol series, and cleared with xylene. Sections treated with normal rabbit serum (1 : 1,000) instead of anti-NF or anti-SP antibodies were used as negative controls. The slides were examined and photographed with a Leitz Dialux 22 microscope equipped with a Vario-Orthomat camera.

Morphometric Evaluation of the Stains The NF and SP positivities were quantitated using a computer-assisted image analysis system, which consisted of an Olympus BH-2 microscope with a color camera attachment (Hitachi Color Camera FP-3060A, Hitachi Denshi, Ldt.) and a Sony Trinitron monitor. Lengths of the positive nerves were measured by moving a cursor on a Hipad Digitizer (Bausch & Lomb, Houston Instrument Div.) and simultaneously following the trace of the cursor on the image of the section, which was projected on the monitor screen. The microscope, the monitor, and the digitizer were connected to an IBM Personal Computer.

One representative section on each slide was examined using a 20x objective. Five adjacent fields were analyzed, which usually covered the whole section. Each field consisted of an approximately equal area of dermis and epidermis. The lengths of the NF and SP positive nerves were measured separately in dermis and epidermis in each specimen.

Statistics The results in each group studied are expressed as the mean ± SEM, and Student's t test has been used in comparison of the means.

RESULTS

With the staining procedure used in the present study, both NF and SP positive fibers could be reproducibly observed in the skin of the three different groups.

The lengths measured of the NF positive fibers both in dermal and epidermal compartments were significantly greater in psoriatic lesions (Group 1) than in lesion-free psoriatic (Group 2) or control (Group 3) skin (Fig 1, Table I).

No significance was recorded in the dermal SP-positivity between different groups. However, the measured epidermal SP-containing nerve fibers summed up significantly higher in psoriatic lesions when compared to the other groups (Fig 2, Table I). No significant differences in any of the measured parameters were obtained between Groups 2 and 3.

In Group 1, the NF positivity was quite frequently seen in the nerve fibers among the dermal inflammatory infiltrate, and in the epidermis as well (Fig. 1*a*). In Groups 2 and 3, NF positive fibers were observed in the dermis but very rarely in the epidermis (Figs 1*b*,*c*).

In Group 1, SP positive nerve fibers were mainly localized in the papillary dermis as short thin strands which occasionally extended into the epidermis (Fig 2). In Groups 2 and 3, faint SP positivities were observed in the dermis, and like NF positivity, very rarely in the epidermis.

DISCUSSION

Evaluation of cutaneous nerves in a histologic section is quite difficult because nerve fibers appear randomly and only partially in a plane of section. Another difficulty is the relatively small number of nerves that can be observed on one section. There are ca. 100 free nerve endings per mm² in a dermal papilla, and only 1–5 in the epidermis of human digital skin [18]. Also, fingertips and toes are much more densely innervated than other skin areas that were used in this study. Presence of SP-containing nerve fibers has been reported in human skin, but they are most abundant in fingers and toes [8]. To overcome these problems in this study relatively thick sections (16 μm) were cut, and the lengths of the stained nerve fibers were measured morphometrically. However, sources of error still exist, e.g., slight variation in section thickness is possible, and the

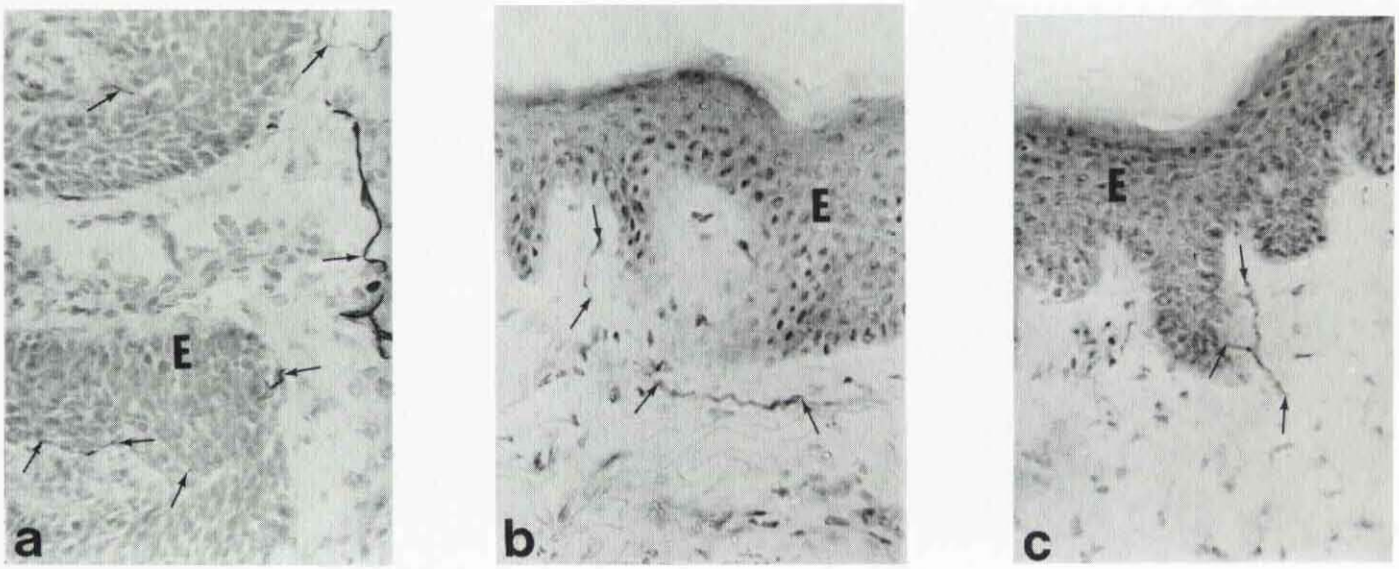

Figure 1. Sensory nerve fibers *(arrows)* in the skin. *E*: epidermis. Anti-neurofilament antibody (x 450). *(a)* Psoriatic lesion: A bundle of nerves can be seen in the dermis, thin fibers also can be seen within the thickened epidermis. *(b)* Lesion-free psoriatic skin. *(c)* Control skin.

Table I. Lengths of the Positive Nerves (±SEM) and Their Significances as Tested against Group 1

Parameter	Group	Length (μm)	p<
NF^{a}_{dermis}	1	833.5 ± 117.8	
	2	344.4 ± 138.0	0.010
	3	530.3 ± 102.4	0.050
$NF^{a}_{epidermis}$	1	156.8 ± 40.2	
	2	25.8 ± 13.3	0.025
	3	29.1 ± 6.9	0.015
SP^{b}_{dermis}	1	260.6 ± 56.3	
	2	118.4 ± 71.1	n.s.[c]
	3	146.4 ± 23.6	n.s.[c]
$SP^{b}_{epidermis}$	1	89.7 ± 16.2	
	2	39.2 ± 8.7	0.035
	3	10.0 ± 5.1	0.001

[a] NF: neurofilament positivity.
[b] SP: substance P positivity.
[c] n.s.: not significant.

fact that only nerve lengths, not thicknesses, were analyzed. SP is rapidly degraded in tissues [19], and thus the time between removal and freezing of the biopsy is critical. It is also practically impossible to obtain biopsies from lesions that are at the same stage, which may produce variation in the results. Regardless, significant differences between the studied groups were found.

Both the dermis and epidermis in psoriatic lesions were significantly more densely innervated with NF-positive fibers when compared to either lesion-free psoriatic or control skin (Table I). The anti-NF antibody stains sensory nerve fibers quite exclusively as has been reported earlier [8,17,20,21]. SP-positivity was scanty in all specimens studied, but there was an increase in the total length of SP-containing fibers in the psoriatic lesion, the increase being significant in the epidermal compartment (Table I). Nerve fibers, including SP-positive ones, occasionally extended to the free surface of the epidermis (Fig 2). A corresponding finding was reported by Weddell et al [15], who stained sections from psoriatic lesions with traditional silver stains. Previous studies have revealed no increase in SP reactivity in psoriatic skin [22,23]. This could be due to a different anti-SP antibody as well as a more sensitive staining system with avidin-biotin amplification used in this study. It is possible that the increased innervation of the psoriatic lesion is secondary to the inflammatory reaction in the dermis with the more prominent development of dermal papillary blood vessels. In fact, the absence of significant alterations in lesion-free skin suggests that there may be a link between the development of a psoriatic plaque and the altered and increased number of sensory nerves in the skin. Based on the current results, it cannot be determined whether the alteration in the nerves precedes the appearance of the lesion or whether it develops at the same time along with the other histologic changes. To test the specificity of the findings in psoriasis, nerve patterns in other inflammatory skin diseases as well as in early psoriatic lesions should be studied. Our preliminary studies on lichen ruber planus biopsies indicate that the innervation and SP-content in these lesions resemble those in normal skin (Naukkarinen et al, in preparation).

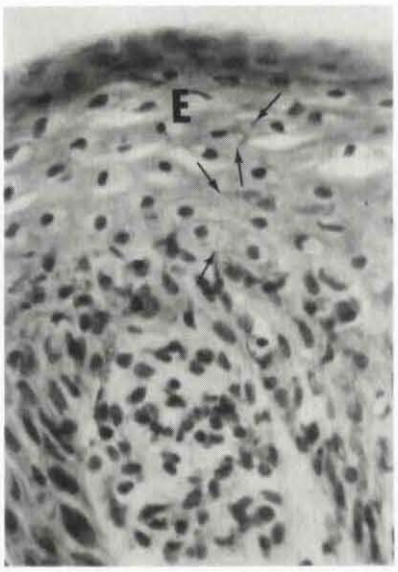

Figure 2. A thin substance P positive nerve fiber *(arrows)*, partially visible, extends through the epidermis *(E)* in a psoriatic lesion. Anti-substance P antibody (x 900).

Recently, SP has been shown to increase the severity of arthritis in human arthritic joints when injected [24], and stimulate synoviocyte proliferation as well as prostaglandin E2 and collagenase release from these cells [25]. Similarly, in psoriatic skin, SP may act as a mediator by binding to the receptors on mast cells inducing degranulation [26], which releases numeral mediators of inflammation [27]. The increased number of mast cells [14] and sensory nerve fibers (Fig 1*a*, Table I) provides an excellent microenvironment for the rapid amplification of neurogenic inflammation through the axon reflex in the psoriatic lesion [5,10]. In this study, the measured SP-positivity was significantly increased in the epidermis of the psoriatic lesion only (Table I). However, SP is not the only neuropeptide detected in the sensory nerve fibers. Coexistence of SP and calcitonin gene-related peptide (CGRP) in these nerves has been observed in rat intestine [28], guinea pig skin [29], and also in human digital skin [8]. Because the psoriatic lesion is densely innervated with sensory fibers, it is possible that other neuropeptides besides SP are contributing to the inflammatory reaction in the lesion [5]. CGRP and neurokinin A have been presented as candidates for mediating neurogenic inflammation [30]. Both of these peptides have characteristics in common with SP. All of them are potent vasodilators and become depleted from the sensory nerves by capsaicin [30], which has been used to treat psoriasis [31].

The potency of SP to influence immunocompetent cells such as macrophages and T lymphocytes, which produce numerous mediators of inflammation, could account for the inflammatory reaction in the psoriatic papillary dermis [32–34]. This suggestion is supported by the observation that strands of SP-positive nerves were detected in the epidermis and in the papillae of the psoriatic lesions surrounded by inflammatory cells. It must be pointed out, however, that with the immunohistochemical techniques used in this study, it is impossible to know if SP is present in the psoriatic lesions in biologically active amounts. SP is difficult to detect because it is easily degraded in tissues [19]. A study by Wallengren et al describes elevated SP concentrations in suction blister fluid of psoriasis as compared to healthy controls as well as certain other inflammatory dermatoses [35].

This study shows that sensory nerve fibers are significantly increased in the psoriatic lesion as compared to lesion-free psoriatic or control skin. SP-content, although scanty and not measured per se, was significantly greater in the epidermal nerve fibers of the psoriatic lesion. These findings suggest that SP, or other neuropeptides in sensory nerves, may be an important mediator in the inflammatory processes associated with the psoriatic lesion.

The authors want to thank Professor Klaus G. Bensch (Department of Pathology, Stanford University) for providing excellent facilities for this work, and Dr. Molly Johnson, Dr. Vera Morhenn (Department of Dermatology, Stanford University), and Dr. Jonathan Mansbridge (Psoriasis Research Institute, Palo Alto, CA) for kindly supplying the biopsy material.

REFERENCES

1. Farber EM, Nall L, Strefling A: Psoriasis: A disease of the total skin. J Am Acad Dermatol 12:150–156, 1985
2. Krueger GG: Psoriasis: Current concepts of its etiology and pathogen-

esis. In: Dobson RI, Triers BH (eds.). Year Book of Dermatology. Year Book Medical Publishers, Chicago, 1981, pp 13–70

3. Payne CMER: Psoriatic science. Br Med J 295:1158–1160, 1987
4. Farber EM, Nall ML: The natural history of psoriasis in 5,600 patients. Dermatologica 148:1–18, 1974
5. Farber EM, Nickoloff BJ, Recht B, Fraki JE: Stress, symmetry, and psoriasis: Possible role of neuropeptides. J Am Acad Dermatol 14:305–311, 1986
6. Pernow B: Substance P. Pharmacol Rev 35:85–141, 1983
7. Dalsgaard C-J, Jonsson C-E, Hokfelt T, Cuello AC: Localization of substance P-immunoreactive nerve fibers in the human digital skin. Experientia 39:1018–1020, 1983
8. Wallengren J, Ekman R, Sundler F: Occurrence and distribution of neuropeptides in the human skin. Acta Derm Venereol (Stockh) 67:185–192, 1987
9. Shanahan F, Denburg JA, Fox J, Bienenstock J, Befus D: Mast cell heterogeneity: effects of neuroenteric peptides on histamine release. J Immunol 135:1331–1337, 1985
10. Lembeck F, Holzer P: Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn-Schmiedeberg's Arch Pharmacol 310:175–183, 1979
11. Brody I: Mast cell degranulation in the evolution of acute eruptive guttate psoriasis vulgaris. J Invest Dermatol 82:460–464, 1984
12. Brody I: Dermal and epidermal involvement in the evolution of acute eruptive guttate psoriasis vulgaris. J Invest Dermatol 82:465–470, 1984
13. Schubert C, Christophers E: Mast cells and macrophages in early relapsing psoriasis. Arch Dermatol Res 277:352–358, 1985
14. Cox AJ: Mast cells in psoriasis. In: Farber EM, Cox AJ (eds.). Psoriasis, proceedings of the second international symposium. Yorke Medical Books, New York, 1976, pp 36–43
15. Weddell G, Cowan MA, Palmer E, Ramaswamy S: Psoriatic skin. Arch Dermatol 91:252–266, 1965
16. Dahl D, Bignami A: Preparation of antisera to neurofilament protein from chicken brain and human sciatic nerve. J Comp Neurol 176:645–648, 1978
17. Dalsgaard CJ, Bjorklund H, Jonsson CE, Hermansson A, Dahl D: Distribution of neurofilament-immunoreactive nerve fibers in human skin. J Histochem 81:111–114, 1984
18. Cauna N: Fine morphological characteristics and microtopography of the free nerve endings of the human digital skin. Anat Rec 198:643–656, 1980
19. Kikuchi M, Fukuyama K: Hydrolysis of neuropeptides by soluble dipeptidyl peptidase IV (DPP IV) purified from rat epidermis. J Invest Dermatol 90:575, 1988
20. Dalsgaard C-J, Bjorklund H, Jonsson C-E, Dahl D: Distribution of neurofilament-immunoreactive nerve fibers in human skin. Histochemistry 81:111–114, 1984
21. Bjorklund H, Dalsgaard C-J, Jonsson C-E, Hermansson A: Sensory and autonomic innervation of non-hairy and hairy human skin. An immunohistochemical study. Cell Tissue Res 243:51–57, 1986
22. Johansson O, Nordlind K: Immunohistochemical screening of psoriasis. Arch Dermatol Res 277:156, 1985
23. Johansson O, Olsson A, Enhamre A, Hammar H, Goldstein M: Phenylethanolamine N-methyl-transferase-like immunoreactivity in psoriasis. An immunohistochemical study on catecholamine synthesizing enzymes and neuropeptides of the skin. Acta Derm Venereol (Stockh) 67:1–7, 1987
24. Levine JD, Clark R, Devor M, Helms C, Moskowitz MA, Basbaum AI: Intraneuronal substance P contributes to the severity of experimental arthritis. Science 226:547–549, 1984
25. Lotz M, Carson DA, Vaughan JH: Substance P activation of rheumatoid synoviocytes: neural pathway in pathogenesis of arthritis. Science 235:893–895, 1987
26. Benyon RC, Lowman MA, Church MK: Human skin mast cells: their dispersion, purification, and secretory characterization. J Immunol 138:861–867, 1987
27. Fraki JE, Schechter NM, Lazarus GS: Human skin proteinases as inflammatory mediators. Br J Dermatol 1095:72–76, 1983
28. Bienenstock J, Tomioka M, Matsuda H, Stead RH: The role of mast cells in inflammatory processes: Evidence for nerve/mast cell interactions. Int Archs Allergy Appl Immunol 82:238–243, 1987
29. Gibbins JL, Furness JB, Costa M, MacIntyre I, Hillyard CJ, Gingis S: Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci Lett 57:125–130, 1985
30. Foreman JC: Peptides and neurogenic inflammation. Br Med Bull 43:386–400, 1987
31. Farber EM, Reusch MK, Glinski W, Karasek MA, Recht B, Nickoloff BJ: Perspectives on psoriasis—The psychoneural pathogenesis of psoriasis. In: Farber EM, Nall L, Morhenn VB, Jacobs PH (eds.). Psoriasis: Proceedings of the 4th International Symposium. Elsevier, New York, 1987, pp 3–9
32. Hartung H-P, Wolters K, Toyka K: Binding of substance P to macrophages: Stimulation of the release of proinflammatory and immunomodulating compounds. Neurosci Lett (suppl) 18:S101, 1984
33. Payan DG, Brewster DR, Goetzl EJ: Specific stimulation of human T lymphocytes by substance P. J Immunol 131:1613–1615, 1983
34. Payan DG, Brewster DR, Missirian-Bustian A, Goetzl EJ: Substance P recognition by a subset of human T lymphocytes. J Clin Invest 74:1532–1539, 1984
35. Wallengren J, Ekman R, Moller H: Substance P and vasoactive intestinal peptide in bullous and inflammatory skin disease. Acta Derm Venereol (Stockh) 66:23–28, 1986